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Predicting harmful algal blooms: a case study with *Dinophysis ovum* in the Gulf of Mexico

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Blooms of *Dinophysis ovum* and *Mesodinium* spp. have been observed in the Gulf of Mexico since 2007 using the Imaging FlowCytobot technology. Bloom dynamics of these two organisms in conjunction with ancillary environmental data for a 5-year period were analyzed to identify the conditions necessary for bloom initiation or presence with the goal of predicting future blooms of *D. ovum*. Using time-series analysis, we observed a positive time-lagged correlation between the two organisms in each year when both were present, which suggests that the presence of *Mesodinium* may be useful as a leading indicator for a *D. ovum* bloom. Although in some cases *D. ovum* and *Mesodinium* co-occurred, no strong predator–prey relationship was observed. We identified a narrow range of temperature and salinity that could be necessary for bloom initiation of *D. ovum* and *Mesodinium* in the Gulf of Mexico. Analysis of images over the time series revealed a wide range in the size of *Mesodinium* cells, which suggests that species other than *M. rubrum* may be present in the Gulf of Mexico. Based on the occurrence of a *D. ovum* bloom preceded by low abundances of *Mesodinium*, we suggest that *D. ovum* is able to utilize ciliates other than *M. rubrum* as prey. Our observations indicate that environmental conditions, as well as *Mesodinium* abundance and species composition, can affect initiation, presence or abundance of *D. ovum* and thus may help in the prediction of future blooms.

KEYWORDS: *Mesodinium rubrum*; dinoflagellate; harmful algae; time series analysis; phytoplankton

INTRODUCTION

Species of the genus *Dinophysis* are distributed worldwide in coastal and oceanic waters and are known to cause harmful algal blooms (Hallegraeff and Lucas, 1988). Recently, this toxic dinoflagellate has been observed blooming in the Gulf of Mexico (Campbell *et al.*, 2010).

Species of *Dinophysis* produce okadaic acid, dinophysistoxins and pectenotoxins, which can cause diarrhetic shellfish poisoning (DSP) in humans (Yasumoto *et al.*, 1985). Mixotrophic species of *Dinophysis* use a peduncle to consume the cell contents of their prey and can maintain photosynthetically active plastids for several generations, enabling growth in the absence of prey (Kim *et al.*, 2008, 2012). Duration of growth in the absence of prey varies among species and can range from 1 week to more than 1 month after feeding (Kim *et al.*, 2008; Nielsen *et al.*, 2012). Survival of *Dinophysis* in the absence of prey can be much longer; it has been reported that some species of *Dinophysis* can survive up to 3 months in the light, but maximum growth (0.40–0.91 divisions day^{-1} at 15–20°C) cannot be maintained (Hansen *et al.*, 2013; Kim *et al.*, 2008; Nielsen *et al.*, 2012). *Mesodinium rubrum* (= *Myrionecta rubra*) has been identified as a prey item for *Dinophysis* when grown with the cryptophyte *Teleaulax* sp. in culture and is the only confirmed species of *Mesodinium* that *Dinophysis* utilizes as prey (Kim *et al.*, 2008, 2012; Nagai *et al.*, 2008; Nishitani *et al.*, 2008, 2010; Park *et al.*, 2006). In a previous study, the maximum ingestion rate of *Mesodinium* by *Dinophysis* was 3.2 cells *Dinophysis*$^{-1}$ day^{-1} (Kim *et al.*, 2008).

Mesodinium rubrum is a non-toxic, mixotrophic ciliate that is globally distributed (Crawford, 1989; Garcia-Cuetos *et al.*, 2012; Johnson and Stoecker, 2005; Johnson *et al.*, 2013). *Mesodinium rubrum* can maintain photosynthetic growth in the absence of prey for several weeks and can survive without prey for several months (Hansen *et al.*, 2013; Myung *et al.*, 2013). It has been proposed that *Mesodinium* availability is one essential condition for a subsequent *Dinophysis* bloom (Diaz *et al.*, 2013). Several culture experiments have reported an increased *Dinophysis* growth rate with an increase in *M. rubrum* availability, showing the dependence of *Dinophysis* on *Mesodinium* (Kim *et al.*, 2008; Riisgaard and Hansen, 2009; Tong *et al.*, 2010). It has also been reported that increased abundances of *M. rubrum* have preceded *Dinophysis* blooms in field studies in several locations (Campbell *et al.*, 2010; Diaz *et al.*, 2013; Minnhagen, 2010; Velo Suarez *et al.*, 2014).

In 2008, a large *D. ovum* bloom occurred in the Gulf of Mexico and early warning was provided using Imaging FlowCytobot (IFCB) images (Campbell *et al.*, 2010; Swanson *et al.*, 2010). This event led to the first closure of shellfish beds and recall of oysters in the USA due to high *D. ovum* abundance and okadaic acid contamination in shellfish. This shutdown of shellfish harvesting occurred shortly before a local annual oyster festival where up to 30 000 people might have been affected by DSP (Campbell *et al.*, 2010; Deeds *et al.*, 2010). Prior to this unexpected *D. ovum* bloom, *Mesodinium spp.* had a period of high abundance. Campbell *et al.* (Campbell *et al.*, 2010) noted a wide range in size of the *Mesodinium* cells seen in IFCB images throughout the course of the bloom. Previously, differences in size of *Mesodinium* cells were attributed to variations in nutrients and prey availability (Montagnes *et al.*, 2008). Recently, Garcia-Cuetos *et al.* (Garcia-Cuetos *et al.*, 2012) compared five species of *Mesodinium* and reported a difference in size among the species.

The IFCB has provided image data of *Mesodinium* and *D. ovum* abundance since the event in 2008. To investigate bloom dynamics of the two organisms, we examined IFCB cell abundance data for 2007–2012 to determine (i) if *Mesodinium*, as prey for *Dinophysis*, can be used as a predictor for a *D. ovum* bloom, (ii) if environmental conditions have an influence on bloom onset or bloom formation of *D. ovum* and *Mesodinium*, and (iii) if differences in *Mesodinium* cell size are evidence of multiple species in the Gulf of Mexico. Results from this study add to our understanding of bloom dynamics of these two organisms and may assist in predicting the occurrence of future *D. ovum* blooms.

METHOD

Sampling region and data acquisition

The IFCB has been deployed at the University of Texas Marine Science Institute (UTMSI) pier laboratory, located on the Port Aransas, TX, USA ship channel (27.84°N, 97.05°W) since September 2007 (Fig. 1). This relatively new imaging system collects real time, near-continuous observations of algal species abundance. The IFCB collects a 5 mL sample from a 4 m depth every 20 min. Combining flow cytometry and video technology, the IFCB is equipped with a red diode laser that causes chlorophyll containing cells to emit red fluorescence and trigger a frame grabber to capture and record images of cells that are within the size range ~10–100 μm (Olson and Sosik, 2007; Sosik and Olson, 2007). A file is produced containing images of the phytoplankton and microzooplankton community and many of these images can be identified to genus or species level (Campbell *et al.*, 2010; Sosik and Olson, 2007). The Port Aransas ship channel is a well-mixed channel with strong tidal currents. Water temperature ranges from 10–37°C (average ~23°C), salinity ranges from ~13–40 (average ~33) and tidal velocity ranges from −1.5–1.8 m s^{-1} where negative values indicate water movement into the channel.

Data classification

IFCB data were processed and classified following the approach described in Sosik and Olson (Sosik and Olson,

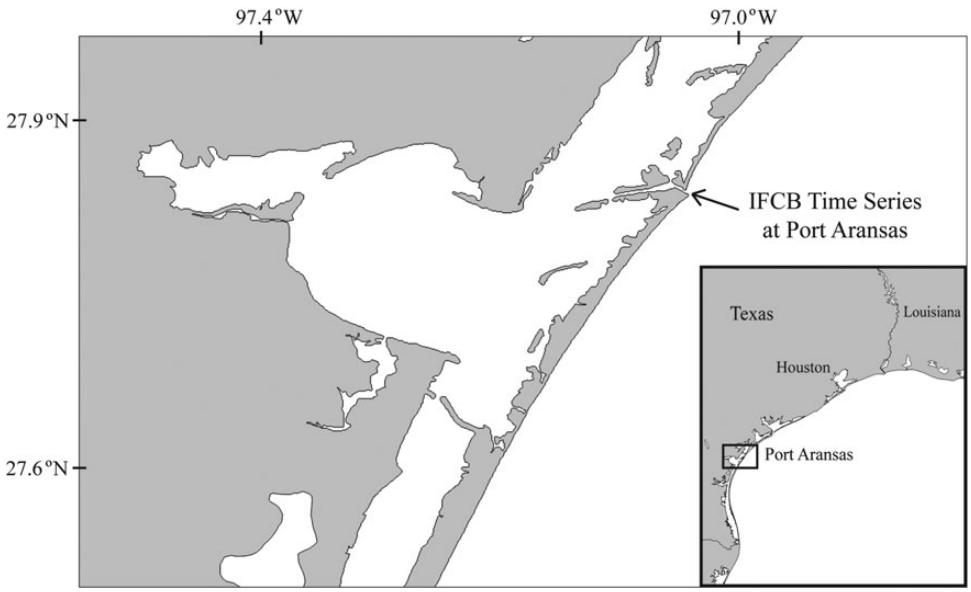

Fig. 1. Location of the Imaging Flow Cytobot (IFCB) in Port Aransas, TX, USA at the University of Texas Marine Science Institute Pier Laboratory (27.84°N, 97.05°W) at the entrance to Corpus Christi Bay.

2007) and Campbell *et al.* (Campbell *et al.*, 2010) with the modification of replacing the support vector machine with the random forest approach described by Breiman (Breiman, 2001). Six automated classifiers were created with the intention to optimize accurate enumeration of the *Dinophysis* and *Mesodinium* categories. A different threshold of classification probability scores was selected for each classifier from the random forest as implemented by the TreeBagger function in MATLAB. The different thresholds selected were the values that gave the least number of residuals between manual (see below) and classifier-estimated abundances.

Each classifier contained 53 categories that were chosen based on the community composition of phytoplankton and microzooplankton seen in the sampling region. Training sets for each category except *Dinophysis* and *Mesodinium* were made up of images spanning the data set from 2007 to 2012. *Dinophysis* and *Mesodinium* training sets were modified to contain only images from 1 year of the data set for each year of the time series (six classifiers total). Each of the six classifiers was applied only to the year corresponding to *Dinophysis* and *Mesodinium* training set images (i.e. 2007 classifier applied only to 2007 data). The classified data were separated into five intervals, each ranging from September to August in order to cover the full blooms of *Mesodinium* and *Dinophysis* (e.g. September 2007–August 2008).

To check the accuracy of each automated classifier, a large number of files (~300–2000) from each year of data were manually corrected. These files were visually inspected and images of *Dinophysis* and *Mesodinium* were manually sorted into their correct categories. A correlation between manual and automated results was computed for each of the five intervals (Supplementary data online, Table SI). By creating a different classifier for each year of data, the correlations of automated results to manual were higher than when one classifier was applied to the entire data set. A correction factor was applied to automated results of *Mesodinium* abundance from 2008 for the 2008/09 interval. By multiplying *Mesodinium* abundance for 1 September—31 December, 2008 by 4.5, the correlation of automated results to manual for the 2008/09 interval was improved. A correction was not required for any other year.

Manually corrected files span the data set from the onset of each bloom to termination in most cases; bloom termination for 2012 was not collected due to an instrument shutdown. Manual results were used to determine bloom initiation times for *Dinophysis* and *Mesodinium*. In this study, background cell abundance is defined as concentration <2 cells mL^{-1} and bloom initiation is defined as the first observation of concentration ≥ 2 cells mL^{-1}, both based on empirical observations of our time series. A bloom is defined as concentration ≥ 5 cells mL^{-1}, based on the legal limit of abundance necessary for the closure of shellfish harvesting for other HAB species as reported by the US Food and Drug Administration (FDA, 2011).

Species identification of *Dinophysis* from the 2008 event was verified using molecular analysis and it was found that the bloom was primarily dominated by *D. ovum* (Campbell *et al.*, 2010). Images of *Dinophysis* from subsequent data

were determined to be *D. ovum* based on visual comparisons to the *Dinophysis* images from 2008. The classifier category for *Dinophysis* also contained images of *D. caudata*, but this genus contributed less than 1% of the total in all bloom years except 2010, in which 17% of the total was *D. caudata* (verified by manual results).

Size analysis

Cell size estimates were calculated from manually inspected IFCB images of *Mesodinium*. The estimated size of each cell was obtained using the cross-sectional area of each image following the method described in Henrichs *et al*. (Henrichs *et al*., 2011). The cross-sectional area was used as a proxy for cell size and will be referred to as cell size throughout. Estimates of *Mesodinium* cell size were used to identify differences in size over the course of each bloom and among years. Approximated cross-sectional area for each *Mesodinium* species was calculated using the length and width ranges given by Garcia-Cuetos *et al*. (Garcia-Cuetos *et al*., 2012) and the equation for the area of an ellipse, given the generalized geometric shape of *Mesodinium*.

Environmental data

Salinity, water temperature and tidal velocity data were downloaded from two stations using the Texas A&M University Corpus Christi Division of Nearshore Research website (http://lighthouse.tamucc.edu). Hourly water temperature and tidal velocity data were obtained from the Real-Time Navigation System Station (RTNS, Station 109) and hourly salinity data were obtained from the Mission Aransas National Estuarine Research Reserve (MANERR #5, Station 149). Both stations are located on the UTMSI pier in Port Aransas. All data were linearly interpolated to replace missing values. A portion of the 2008 salinity record is questionable with unexplained decreases on a 2-week frequency interval. This is not expected to interfere with results from this study; bloom initiation of *D. ovum* and *Mesodinium* did not coincide with the questionable data.

Wind data were downloaded from the National Oceanic and Atmospheric Administration (NOAA) National Data Buoy Center (NDBC) website (http://ndbc.noaa.gov) for station PTAT2, which is located near the Port Aransas ship channel (27.828°N 97.050°W). Wind speed and direction were downloaded and transformed following the method described by Ogle (Ogle, 2012). The east/west and north/south components of the wind data were separated and rotated with respect to the angle of the coast in order to get the along shore component of the wind. Monthly averages of the along shore wind component were calculated and used as a proxy for Ekman transport toward the shore and downwelling strength.

Statistical analysis

Statistical analysis was performed using MATLAB Statistics Toolbox (MATLAB R2011, The MathWorks Inc.). All data were tested for normality; automated cell abundance data were not normally distributed and were $\log(x+1)$ transformed prior to time-series analysis, where x = cells mL^{-1}, in order to account for abundances with a value of zero throughout the time series. Time series of temperature, salinity and cell abundance were compared using time-lagged correlations to observe the interannual relationship between cell abundance and environmental variables. These time series were put into standard form prior to analysis (i.e. de-meaned and divided by the standard deviation). A maximum lag of 2000 h (~83 days) was chosen for the time-lagged correlations in order to focus on the most influential time period surrounding the blooms of *D. ovum* and *Mesodinium*. Because the time series of *D. ovum* and *Mesodinium* abundance were non-stationary, significance for all computed correlations was obtained after degrees of freedom were calculated (Emery and Thomson, 2001). ANOVA and the Tukey–Kramer honestly significant difference procedure were used to determine differences among years of *Mesodinium* cell sizes. These data were found to be log normally distributed and were log transformed prior to the ANOVA.

RESULTS

Cell abundance and bloom timing

Dinophysis ovum blooms occurred in 4 of the 5 years of the time series: 2007/08, 2009/10, 2010/11 and 2011/12 (Fig. 2). *Mesodinium* blooms also occurred in 4 of the 5 years: 2007/08, 2008/09, 2009/10 and 2010/11 (Fig. 2). The 2007/08 blooms of *D. ovum* and *Mesodinium* had the highest abundance reaching peaks of ~200 and ~300 cells mL^{-1}, respectively (Supplementary data online, Table SII). The highest abundance of *Mesodinium* occurred in late January and the highest abundance of *D. ovum* occurred about 1 month later, in late February. In later years, cell abundances of *D. ovum* and *Mesodinium* were lower and never reached concentrations comparable to the 2007/08 event. In 2008/09, although *Mesodinium* was present above bloom concentration, *D. ovum* cell concentration remained below 1 cell mL^{-1} for the entire year. In 2009/10, the peak in abundance of

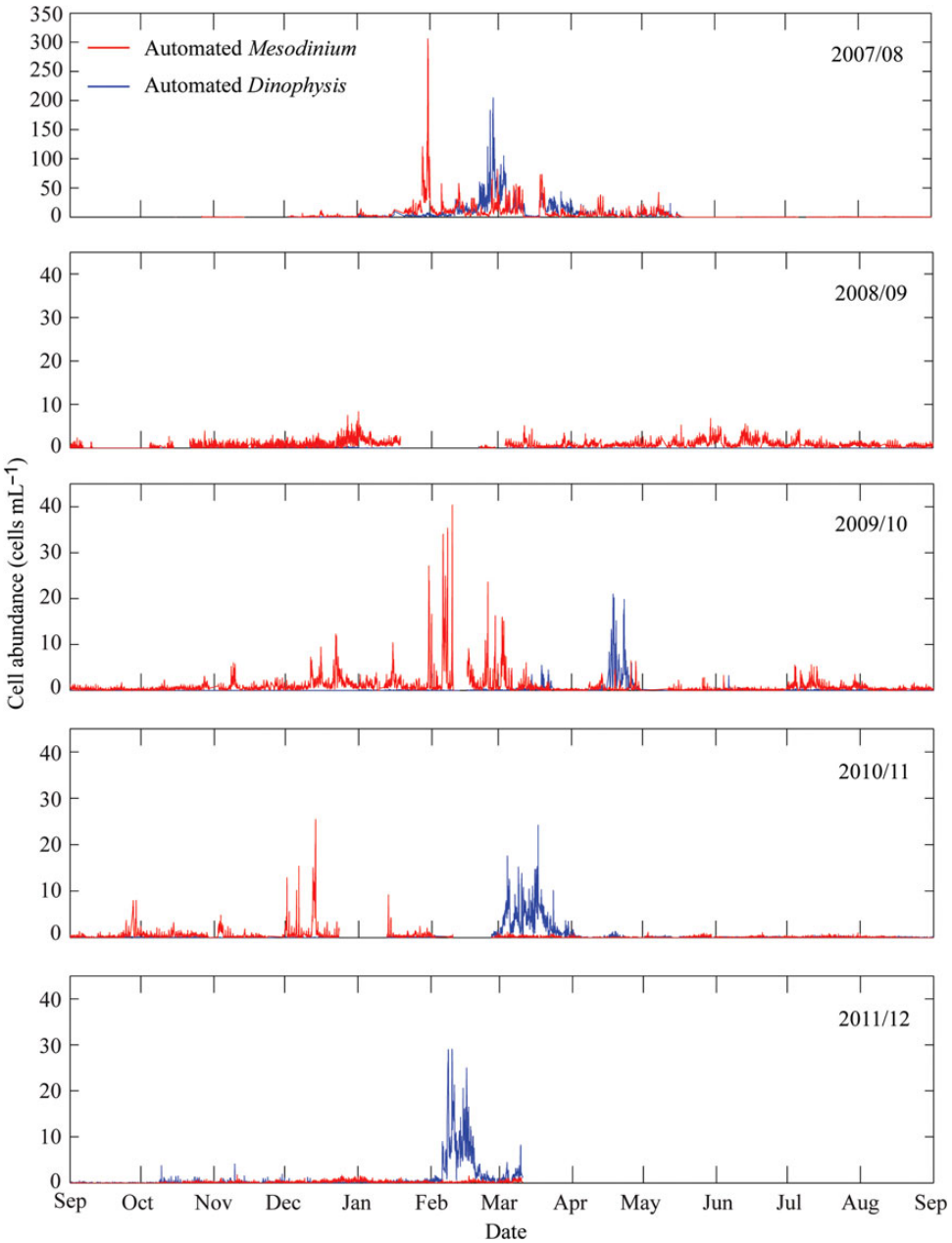

Fig. 2. Time series of *Dinophysis* and *Mesodinium* at Port Aransas, TX, USA (27.84°N, 97.05°W). Automated results for *Dinophysis* are in blue and *Mesodinium* in red. Note difference in scale in 2007/08. Gaps in data from 2008/09, 2009/10 and 2010/11 were due to instrument shut down for repair and preventative maintenance.

Mesodinium occurred in early February and cell concentration fluctuated above 10 cells mL^{-1} until the end of April. The *D. ovum* peak in abundance occurred in mid-April, which was ~2.5 months after the highest peak in abundance of *Mesodinium*. In 2010/11, the highest peak in abundance of *Mesodinium* occurred in mid-December, but cell counts remained above 10 cells mL^{-1} through mid-January. The *D. ovum* peak in abundance occurred 2 months later in mid-March. In 2011/12, although *Mesodinium* was present above background levels, it did not reach bloom concentrations prior to the *D. ovum* bloom, which reached the highest peak in abundance in early February.

Mesodinium blooms occurred between mid-September and May. Correlations between *Mesodinium* abundance of bloom years with temperature and salinity were not

significant. Most blooms of *Mesodinium* corresponded to temperature and salinity values that were below the inter-quartile range (25th−75th percentiles) of their distribution (Fig. 3A). Bloom initiation of *Mesodinium* ranged from mid-September to the end of October (Supplementary data, Table SIII) when temperature and salinity ranged from ∼23−29°C and ∼30−34, respectively. Bloom initiation coincided with an incoming tide in each year except 2009/10. A bloom initiation date for *Mesodinium* could not be identified for 2008/09 because cell concentrations continued to fluctuate above the 2 cells mL^{-1} threshold after the large 2007/08 bloom until the end of the 2008/09 bloom (Fig. 2).

Dinophysis ovum blooms occurred between the end of January and the end of May. Correlations of *D. ovum* abundance of bloom years with temperature and salinity were not significant. Most blooms of *D. ovum* corresponded to temperature and salinity values that were within or slightly below the inter-quartile range of their distribution (Fig. 3B). Bloom initiation of *D. ovum* ranged from the end of January to mid-March (Supplementary data online, Table SIII) when temperature and salinity ranged from ∼11−19°C and ∼28−33, respectively. Bloom initiation occurred on, or just after, an incoming tide each year, with the exception of 2009/10, when velocity =0 after the incoming tide.

Time-series analysis

Time-lagged cross correlations are used to help determine whether one variable can be used as a leading indicator of another. We found a positive trend in correlations with positive lag between *D. ovum* and *Mesodinium* abundance each year except in 2008/09 when *D. ovum* was not present (Table I; Fig. 4). The time lag for the highest positive correlation values ranged from 46 to 62 days, and the correlation coefficients ranged from $r = 0.38-0.50$ ($P < 0.01$).

There was a negative pattern of correlations between *D. ovum* abundance and temperature at zero lag each year except in 2009/10, but the correlations were not significant. Correlations between *D. ovum* abundance and salinity were negative at zero lag each year, but were not significant. There was a negative pattern of correlation for *Mesodinium* abundance with temperature and salinity at zero lag each year except 2008/09, but the correlation was only significant in 2009/10 ($P < 0.05$). Results showed a positive trend correlation between temperature

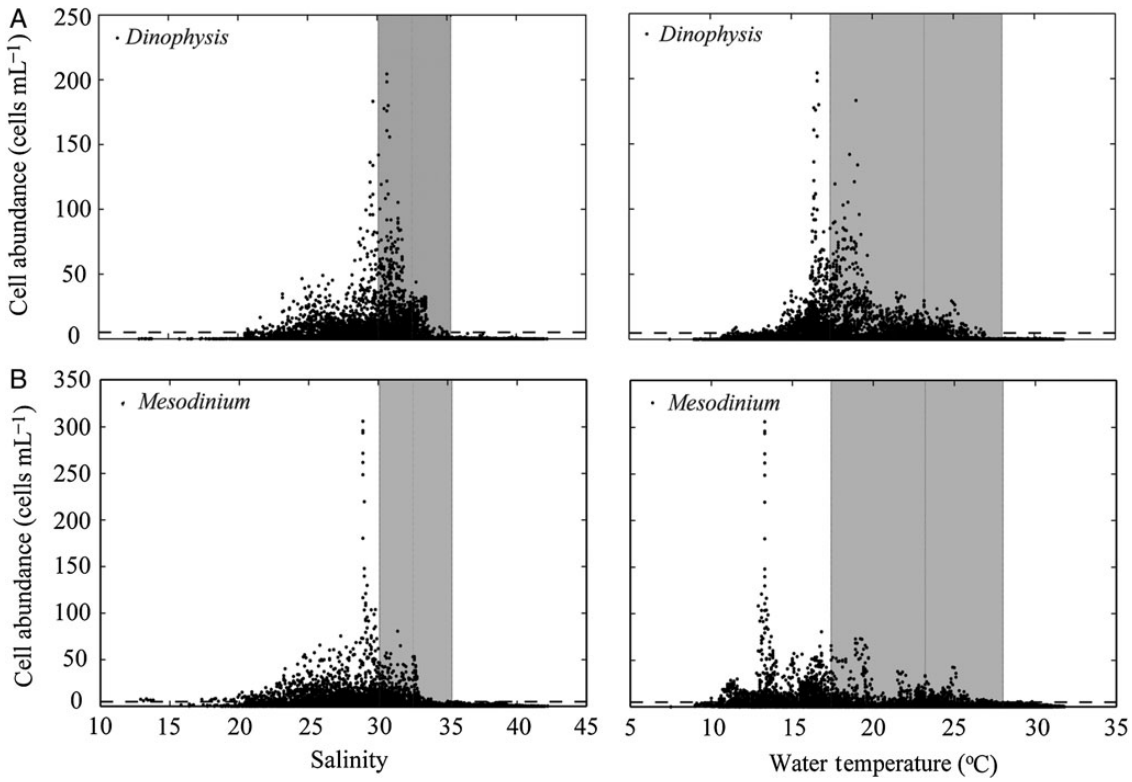

Fig. 3. Water temperature and salinity values plotted with (**A**) *Dinophysis* and (**B**) *Mesodinium* abundance. The solid block represents the 25th−75th percentile range of water temperature and salinity. Note the difference in scale for *Dinophysis* and *Mesodinium* abundance.

and salinity for most lag phases every year, but the correlations were not significant.

Size analysis

The cross-sectional area of *Mesodinium* cells ranged from 224 to 4415 μm^2 (Fig. 5). Using cross-sectional area as a proxy for cell size, average *Mesodinium* cell size was greatest in 2007/08 and lowest in 2008/09 with values 2094 and 731 μm^2, respectively. There was a wide range in *Mesodinium* cell size throughout the course of each bloom and among years (Supplementary data online, Fig. S1). The widest range in sizes occurred in 2007/08 (~283–4415 μm^2) and the smallest range occurred in 2011/12 (~224–2433 μm^2). *Mesodinium* average cell sizes were significantly different in each year of the time series (Supplementary data online, Fig. S2).

DISCUSSION

Cell abundance and bloom timing

High-resolution abundance data provided by the IFCB enabled us to examine the relationship between the harmful algal bloom species *D. ovum* and its ciliate prey. Results from this time series have shown that *Mesodinium* bloomed prior to *D. ovum* each year except 2011/12, when *Mesodinium* was present but did not exceed our defined bloom threshold concentration. These observations provide evidence that *Mesodinium* availability may be necessary for the formation of a *D. ovum* bloom, as suggested by recent studies (Diaz *et al.*, 2013), and it is possible that the presence of *Mesodinium* can be used as a predictor for *D. ovum* blooms. However, results from this study provide a complex picture and the relationship between the two organisms is unclear at this time.

The ratio of prey to predator necessary for a bloom in our region is not yet known. We suggest that bloom concentrations of *Mesodinium* each year were related to the bloom concentration of *D. ovum*, except in 2011/12. A growth rate using *Mesodinium* abundance was calculated using the Michaelis–Menton parameters and the assumptions described by Kim *et al.* (Kim *et al.*, 2008). Growth rates for *D. ovum* in each year, except 2007/08, were less than 0.01 day^{-1}. In 2007/08, the calculated growth rate was 0.34 day^{-1}, which indicates that the abundance of *Mesodinium* may have been sufficient to support the subsequent *Dinophysis* bloom. The largest bloom of both species and the shortest lag between peaks also occurred in this year, which may suggest that the bloom of *Dinophysis* in 2007/08 was caused by the preceding bloom of *Mesodinium*. This calculated growth rate is similar to a previous study in which the growth for the *Dinophysis* bloom in 2007/08, calculated using the frequency of dividing cells, was found to be 0.2–0.3 day^{-1} (Campbell *et al.*, 2010). It is important to note that *Mesodinium* is highly motile and high abundance may be partially caused by aggregation. The IFCB, which sampled at a single depth, may have over- or underestimated abundance. This could account for observations of prey–predator mismatch in our data set. We note also that fluctuation in abundance may be caused by physical concentration of cells at the coast and may not reflect cell growth (Hetland and Campbell, 2007; Thyng *et al.*, 2013).

We observed a wide range in the timing of bloom initiation for both *Mesodinium* (09/19–11/10) and *D. ovum* (01/20–03/14). Temperature and salinity values during *D. ovum* bloom initiation were narrow (~11–19°C and ~28–33, respectively), and it is possible that these conditions are favorable for the formation of a bloom in the Gulf of Mexico. Similarly, there was a narrow range of temperature and salinity during bloom initiation periods for *Mesodinium* bloom years (~23–29°C and ~30–34, respectively). In 2011/12, *Mesodinium* was present above 2 cells mL^{-1}, and bloom initiation was observed, but a bloom (≥5 cells mL^{-1}) never developed. Temperature during bloom initiation for 2011/12 was lower than other years (20°C) and salinity was higher than other years (36). We propose that a temperature range of 23–29°C and a salinity range of 30–34 may be favorable for *Mesodinium* bloom formation in our region and given

Table I: Strongest correlations with lag from the time-series analysis

Time Interval	D–M	D–T	D–S	M–T	M–S	T–S
2007/08	0.78 (0)	−0.71 (31)	−0.45 (0)	−0.69 (20)	−0.55 (0)	0.67 (−23)
2008/09	0.10 (−17)*	−0.33 (24)	−0.26 (−56)	0.22 (−61)*	0.21 (−60)**	0.56 (−38)
2009/10	0.38 (62)**	0.31 (−77)**	−0.25 (50)*	−0.45 (−4)	−0.51 (0)*	0.66 (0)
2010/11	0.38 (51)**	−0.60 (27)*	0.26 (78)	−0.43 (−32)*	−0.33 (0)	0.46 (0)
2011/12	0.50 (46)**	−0.47 (47)	−0.56 (0)	−0.55 (0)	−0.44 (3)	0.77 (−1)

Lag (in days) of correlation in parenthesis.
D, *Dinophysis*; M, *Mesodinium*; T, Water Temperature; S, Salinity.
*$P < 0.05$.
**$P < 0.01$.

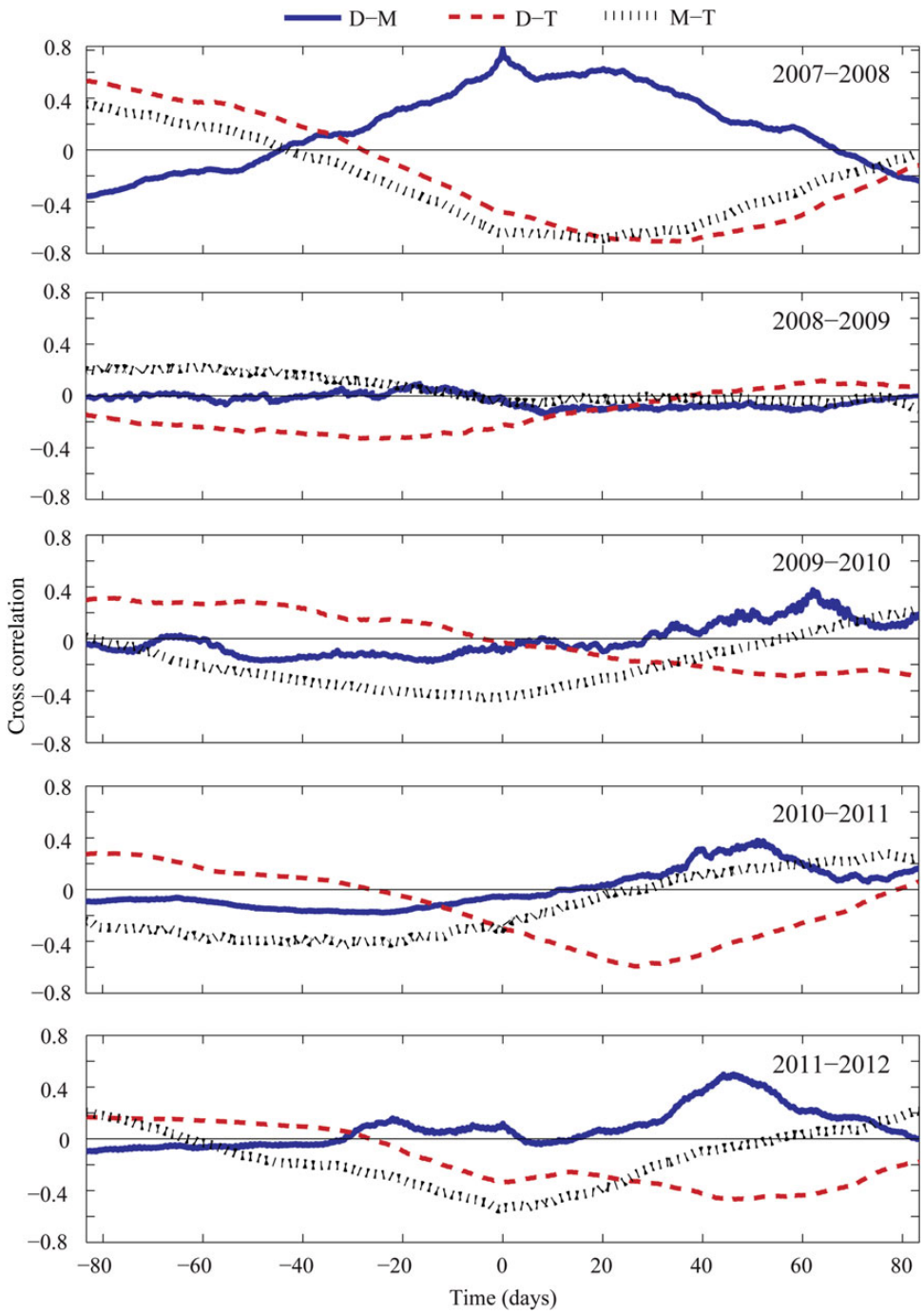

Fig. 4. Cross correlations of automated cell abundance of *Dinophysis* and *Mesodinium* with water temperature and cross correlation of *Dinophysis* with *Mesodinium*. D, *Dinophysis*; M, *Mesodinium*; T, water temperature. See online supplementary data for a color version of this figure.

that the temperature and salinity values were outside of this range in 2011/12, a bloom did not occur. Nevertheless, additional years of data will be needed to confirm this explanation for the absence of a *Mesodinium* bloom in 2011/12. The temperature ranges observed during blooms of *D. ovum* and *Mesodinium* are comparable to previous field and culture studies in more temperate regions (Hansen *et al.*, 2013; Johnson *et al.*, 2013). The salinity ranges observed are similar to many culture studies, but are higher than prior field observations (Johnson *et al.*, 2013; Kim *et al.*, 2012; Park *et al.*, 2008; Yih *et al.*, 2013).

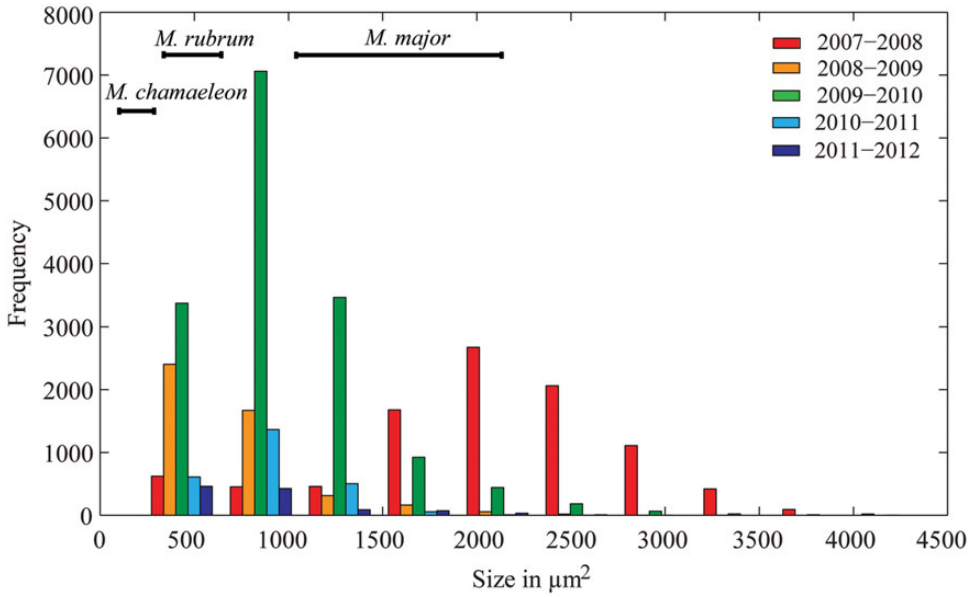

Fig. 5. Histogram of *Mesodinium* cell sizes for each bloom interval. Black bars represent area estimates using size ranges from Garcia-Cuetos *et al.* (Garcia-Cuetos *et al.*, 2012) for *M. rubrum, M. major, M. chamaeleon*. See online supplementary data for a color version of this figure.

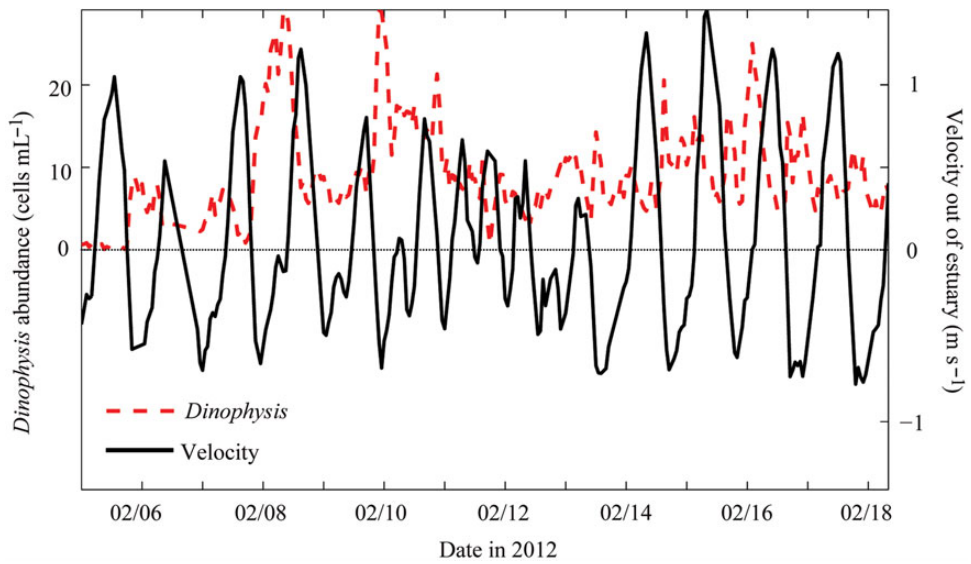

Fig. 6. *Dinophysis* abundance plotted with tidal velocity for a 2-week period in February 2012. Negative values of velocity indicate water movement into the estuary. See online supplementary data for a color version of this figure.

We found that in most cases (except 2010/11), *Mesodinium* and *Dinophysis* bloom initiation occurred during or just after an incoming tide. Cell concentrations increased during incoming tide in many cases (Fig. 6), which confirms previous observations (Campbell *et al.*, 2010) and leads us to the conclusion that the blooms are originating offshore before they are seen in the Port Aransas ship channel. Recently, Ogle (Ogle, 2012) proposed that wind speed and direction along the Texas coast affects the occurrence of blooms in our sampling region. More specifically, the along-shore wind component (used as an indicator for upwelling/downwelling strength of the coastal circulation) for September was related to bloom presence for *Karenia brevis*, a harmful algal bloom species that typically initiated in late September-mid October. We compared the monthly

average along shore wind component for bloom initiation periods of *Dinophysis* and *Mesodinium* to determine if downwelling strength (Ekman transport toward the shore) was related to bloom presence. We found that strong downwelling (i.e. Ekman transport toward the shore) occurred during bloom initiation times for all bloom years of *Mesodinium* and weak downwelling occurred during the non-bloom year (Supplementary data online, Table SIV). It is possible that a *Mesodinium* bloom occurred offshore in 2011/12, but due to weak downwelling the bloom was not transported into the ship channel, thus no bloom was recorded. These observations reinforce the idea that blooms of *Mesodinium* originate offshore and are brought into the ship channel by the currents and incoming tide. Similarly, the along shore wind component during the bloom initiation period for *Dinophysis* was examined, but no difference in wind pattern were observed between bloom and non-bloom years.

We observed that the 2 years with the highest annual mean salinities correspond to the years with the lowest *Mesodinium* peak size and abundance. Similarly, we found that the year with the highest annual temperature mean corresponded to the only year with no *Mesodinium* bloom (data not shown). More observations are needed to determine whether significantly higher values of salinity and temperature over the course of a bloom can be factors for decreased *Mesodinium* abundance.

Time-series analysis

A short lag between peaks and overlap of the two organisms appear to be key factors for the formation of a large bloom of *D. ovum*. A positive correlation between *D. ovum* and *Mesodinium* was observed at different time lags in every year except 2008/09, when a *D. ovum* bloom did not occur. Although *Mesodinium* remained below bloom concentration in 2011/12, a significant positive correlation between *D. ovum* and *Mesodinium* was still present. The time lag for highest correlation corresponded to lag between peaks of the blooms of *D. ovum* and *Mesodinium* and typically ranges from ~1 to 2 months. This is relevant to culture studies that show the ability of some *Dinophysis* species to continue photosynthetic growth without food for periods longer than 1 month (Nielsen *et al.*, 2012; Park *et al.*, 2008). The longest lag (62 days) occurred in 2009/10 and is associated with the highest peaks in abundance of *Mesodinium* and *D. ovum* for this interval. A 2-month lag between blooms is quite long, but *Mesodinium* abundance remained well above background levels after its highest peak and increased above 15 cells mL^{-1} several times before *D. ovum* bloomed. The shortest lag and highest correlation occurred in 2007/08 and is associated with the largest blooms of the time series. The lag for highest correlation in this year is zero due to the overlap of the two organisms. A second peak in correlation occurs at ~20 days and corresponds to the lag between the highest peak of *D. ovum* and *Mesodinium*. It is important to note that *Mesodinium* abundance remained well above background levels and reached abundances >20 cells mL^{-1} throughout the course of the *D. ovum* bloom in 2007/08, which we believe to be significant. Although *Mesodinium* and *D. ovum* overlapped in other years, the abundance of both species was much lower than in 2007/08. In a recent study, fluctuation of *M. rubrum* abundance was closely correlated to *D. acuminata* abundance with a 7-day lag (Yih *et al.*, 2013). The lags found in our study are, in most cases, much longer and may not conclusively prove that *Mesodinium* caused subsequent *D. ovum* blooms.

Time-series analysis of salinity and temperature with cell abundance data were used to investigate why a *D. ovum* bloom did not occur in 2008/09. The cross-correlation patterns for salinity and temperature were similar in each year except 2008/09. The correlations of environmental variables with *D. ovum* were different in 2008/09 because no bloom occurred, but this does not explain why the correlation patterns of environmental variables and *Mesodinium* were different in this year. In every year apart from 2008/09, there was a negative trend in correlation between *Mesodinium* abundance with salinity and temperature at zero lag. In 2008/09, there was no correlation for either pair at zero lag. Although the bloom in 2008/09 was the smallest bloom of *Mesodinium*, low abundance does not seem to be a factor since the negative correlation was seen in 2011/12, the year with the lowest *Mesodinium* abundance. More data are needed to determine whether this anomaly in 2008/09 is significant.

Size analysis

The cross-sectional areas of *Mesodinium* cells seen in the IFCB images ranged from ~225 to ~4400 μm^2. According to Garcia-Cuetos *et al.* (Garcia-Cuetos *et al.*, 2012), length and width of *M. rubrum* range from 25–35 and 16–25 μm, respectively, giving an approximated cross-sectional area range ~315–685 μm^2. Although many of the cross-sectional areas obtained in this study fall within the size range of *M. rubrum*, cells with smaller and larger areas were seen in every year (Fig. 5). The largest species reported, *M. major*, ranges in length 40–55 μm and in width 35–50 μm giving an approximated cross-sectional area ranging ~1100–2160 μm^2. The smallest species reported, *M. chamaeleon*, ranges 19–25 μm in length and 13–17 μm in width giving an approximated cross-sectional area ranging ~195–335 μm^2

(Garcia-Cuetos *et al.*, 2012). These three size classes account for a large majority of our results. Variations in cell area were previously attributed to nutrient and prey availability and all cells were assumed to be *M. rubrum* regardless of size (Montagnes *et al.*, 2008). The wide range in sizes of the different *Mesodinium* species presented by Garcia-Cuetos *et al.* (Garcia-Cuetos *et al.*, 2012) and our observations suggest that the variation in cell area could be associated with multiple species of *Mesodinium* in the Gulf of Mexico. Note that *M. chamaeleon* is a benthic species and is unlikely to be seen in our data set. The observed *Mesodinium* within the *M. chamaeleon* size range is most likely a different small species. Molecular analysis would be necessary to confirm the species identification, and should be addressed in future studies.

In laboratory studies, the only confirmed species of *Mesodinium* that *Dinophysis* utilizes as prey is *M. rubrum* (Hansen *et al.*, 2013; Kim *et al.*, 2008; Minnhagen *et al.*, 2011; Nishitani *et al.*, 2008, 2010; Park *et al.*, 2006). The presence of multiple species of *Mesodinium* in the Gulf of Mexico could be a cause for varying abundance of *D. ovum* in our time series. As it is not certain which species or size range is preferable to *D. ovum*, it is possible that a portion of the *Mesodinium* cells in a bloom are not utilized by *D. ovum* as prey.

In 2007/08, the majority of *Mesodinium* cells were larger than the *M. rubrum* size range (90% of cells were larger). Because this was the year of the largest *D. ovum* bloom, it is possible that *D. ovum* favors other, larger species of *Mesodinium*. This is one explanation for why *D. ovum* did not bloom in 2008/09, even though *Mesodinium* was present. Average cross-sectional area of *Mesodinium* cells in 2008/09 was much smaller than in 2007/08 and although the majority were within the *M. rubrum* size range (78 compared with 10% in 2008), there were very few larger cells (Supplementary data online, Fig. S1).

In 2011/12, the majority of cells were within the *M. rubrum* size range (72%), but abundance was low. A *D. ovum* bloom still occurred in this year, meaning that the *D. ovum* must have obtained enough prey to grow to bloom concentrations. One possible explanation is that *D. ovum* ingested most of the *Mesodinium* offshore and thus no bloom was seen in our samples, but this did not occur in any other year. It has been suggested that *Dinophysis* spp. may feed on other marine ciliates such as *Laboea*, *Tontonia* and *Strombidinium* due to their ability to acquire plastids from many different algal groups including the cryptophyte genus *Teleaulax*. Based on the maximum ingestion rate of *Dinophysis* on *Mesodinium* from a previous study (3.2 cells *Dinophysis*$^{-1}$ day^{-1}), the abundance of *Mesodinium* in our samples would not sustain maximum growth of *Dinophysis* (0.91 day^{-1}) in most cases due to the small number of instances that *Dinophysis* and *Mesodinium* co-occurred (Kim *et al.*, 2008). This suggests that *Dinophysis* is able to utilize ciliates other than *Mesodinium* as prey. Evidence of *Dinophysis* feeding on other ciliates has not been found, but it has been reported that some species contain plastids of several different microalgal origins, implying that *Dinophysis* can utilize other ciliates as prey (Kim *et al.*, 2012; Nishitani *et al.*, 2012). We propose that this may be the case for 2011/12, when the *D. ovum* bloom was not preceded by a *Mesodinium* bloom. Abundance of ciliate groups other than *Mesodinium* were not analyzed in this study but should be considered in future studies.

From this study, it appears that the presence of *Mesodinium* may be useful as a predictor for subsequent *D. ovum* blooms, but at this time we cannot say conclusively whether *M. rubrum* is necessary to initiate or sustain *D. ovum* blooms. We suggest that the temperature and salinity ranges observed during *D. ovum* and *Mesodinium* bloom initiation in bloom years may be ideal conditions for bloom formation in the Gulf of Mexico. Differences in the *Mesodinium* cross-sectional areas observed across years of the time series could indicate different *Mesodinium* species, but molecular analysis for species identification is needed for confirmation. Finally, based on observations of a *D. ovum* bloom preceded by very low abundances of *Mesodinium*, we propose that *D. ovum* is able to utilize ciliates other than *M. rubrum* as prey. Direct evidence of this has not yet been reported, but future studies should include analysis of other ciliate groups prior to *D. ovum* bloom events.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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REFERENCES

Breiman, L. (2001) Random forests. *Mach. Learn.*, **45**, 5–32.

Campbell, L., Olson, R. J., Sosik, H. M. *et al.* (2010) First harmful *Dinophysis* (*Dinophyceae, Dinophysiales*) bloom in the U.S. is revealed by automated imaging flow cytometry. *J. Phycol.*, **46**, 66–75.

Crawford, D. W. (1989) Mesodinium rubrum—the phytoplankter that wasn't. *Mar. Ecol. Prog. Ser.*, **58**, 161–174.

Deeds, J. R., Wiles, K., Heideman VI, G. B. *et al.* (2010) First U.S. report of shellfish harvesting closures due to confirmed okadaic acid in Texas Gulf coast oysters. *Toxicon*, **55**, 1138–1146.

Diaz, P. A., Reguera, B., Ruiz-Villarreal, M. *et al.* (2013) Climate variability and oceanographic settings associated with interannual variability in the initiation of *Dinophysis acuminata* blooms. *Mar. Drugs*, **11**, 2964–2981.

Emery, W. J. and Thomson, R. E. (2001) *Data Analysis Methods in Physical Oceanography*. Elsevier.

Food and Drug Administration (FDA). (2011) National shellfish sanitation program(NSSP): Guide for the control of molluscan shellfish. U.S. Department of Health and Human Services, 2011 revision.

Garcia-Cuetos, L., Moestrup, Ø. and Hansen, P. J. (2012) Studies on the genus Mesodinium II. Ultrastructural and molecular investigations of five marine species help clarifying the taxonomy. *J. Eukaryot. Microbiol.*, **59**, 374–400.

Hallegraeff, G. M. and Lucas, I. A. N. (1988) The marine dinoflagellate genus *Dinophysis* (*Dinophyceae*)—photosynthetic, neritic and non-photosynthetic, oceanic species. *Phycologia*, **27**, 25–42.

Hansen, P. J., Nielsen, L. T., Johnson, M. *et al.* (2013) Acquired phototrophy in *Mesodinium* and *Dinophysis*—a review of cellular organization, prey selectivity, nutrient uptake and bioenergetics. *Harmful Algae*, **28**, 126–139.

Henrichs, D. W., Sosik, H. M., Olson, R. J. *et al.* (2011) Phylogenetic analysis of *Brachidinium capitatum* (*Dinophyceae*) from the Gulf of Mexico indicates membership in the *Kareniacae*. *J. Phycol.*, **47**, 366–374.

Hetland, R. D. and Campbell, L. (2007) Convergent blooms of *Karenia brevis* along the Texas coast. *Geophys. Res. Lett.*, **34**, L19604.

Johnson, M. D. and Stoecker, D. K. (2005) Role of feeding in growth and photophysiology of *Myrionecta rubra*. *Aquat. Microb. Ecol.*, **39**, 303–312.

Johnson, M. D., Stoecker, D. K. and Marshall, H. G. (2013) Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay. *J. Plankton Res.*, **35**, 877–893.

Kim, M., Nam, S., Shin, W. *et al.* (2012) *Dinophysis caudata* (*Dinophyceae*) sequesters and retains plastids from the mixotrophic ciliate prey *Mesodinium rubrum*. *J. Phycol.*, **48**, 569–579.

Kim, S., Kang, Y., Kim, H. *et al.* (2008) Growth and grazing responses of the mixotrophic dinoflagellate *Dinophysis acuminata* as functions of light intensity and prey concentration. *Aquat. Microb. Ecol.*, **51**, 301–310.

Minnhagen, S. (2010) Kleptoplasty in *Dinophysis* spp: Ecological role and evolutionary implications. Linnaeus University Dissertations No 19/2010.

Minnhagen, S., Kim, M., Salomon, P. *et al.* (2011) Active uptake of kleptoplastids by *Dinophysis caudata* from its ciliate prey Myrionecta rubra. *Aquat. Microb. Ecol.*, **62**, 99–108.

Montagnes, D. J. S., Allen, J., Brown, L. *et al.* (2008) Factors controlling the abundance and size distribution of the phototrophic ciliate *Myrionecta rubra* in open waters of the North Atlantic. *J. Eukaryot. Microbiol.*, **55**, 457–465.

Myung, G., Kim, H. S., Park, J. W. *et al.* (2013) Sequestered plastids in *Mesodinium rubrum* are functionally active up to 80 days of phototrophic growth without cryptomonad prey. *Harmful Algae*, **27**, 82–87.

Nagai, S., Nitshitani, G., Tomaru, Y. *et al.* (2008) Predation by the toxic dinoflagellate *Dinophysis fortii* on the ciliate *Myrionecta rubra* and observation of sequestration of ciliate chloroplasts. *J. Phycol.*, **44**, 909–922.

Nielsen, L. T., Krock, B. and Hansen, P. J. (2012) Effects of light and food availability on toxin production, growth and photosynthesis in Dinophysis acuminata. *Mar. Ecol-Prog Ser.*, **471**, 37–50.

Nishitani, G., Nagai, S., Baba, K. *et al.* (2010) High-level congruence of *Myrionecta rubra* prey and *Dinophysis* species plastid identities as revealed by genetic analyses of isolates from Japanese coastal waters. *Appl. Environ. Microb.*, **76**, 2791–2798.

Nishitani, G., Nagai, S., Hayakawa, S. *et al.* (2012) Multiple plastids collected by the dinoflagellate *Dinophysis mitra* through kleptoplastidy. *Appl. Environ. Microb.*, **78**, 813–821.

Nishitani, G., Nagai, S., Sakiyama, S. *et al.* (2008) Successful cultivation of the toxic dinoflagellate *Dinophysis caudata* (*Dinophyceae*). *Plankton Benthos Res.*, **3**, 78–85.

Ogle, M. (2012) Physical mechanisms driving harmful algal blooms along the Texas coast. MS thesis. Texas A&M University, College Station, TX.

Olson, R. J. and Sosik, H. M. (2007) A submersible imaging-in-flow instrument to analyze nano-and microplankton: imaging FlowCytobot. *Limnol. Oceanogr. Methods*, **5**, 195–203.

Park, M., Kim, S., Kim, H. *et al.* (2006) First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquat. Microb. Ecol.*, **45**, 101–106.

Park, M. G., Park, J. S., Kim, M. *et al.* (2008) Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *J. Phycol.*, **44**, 1154–1163.

Riisgaard, K. and Hansen, P. J. (2009) Role of food uptake for photosynthesis, growth and survival of the mixotrophic dinoflagellate *Dinophysis acuminata*. *Mar. Ecol. Prog. Ser.*, **381**, 51–62.

Sosik, H. M. and Olson, R. J. (2007) Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. *Limnol. Oceanogr.*, **5**, 204–216.

Swanson, K., Flewelling, L., Byrd, M. *et al.* (2010) The 2008 Texas *Dinophysis ovum* bloom: distribution and toxicity. *Harmful Algae*, **9**, 190–199.

Thyng, K. M., Hetland, R. D., Ogle, M. T. *et al.* (2013) Origins of *Karenia brevis* harmful algal blooms along the Texas coast. *Limnol. Oceanogr Fluids Environ.*, **3**, 269–278.

Tong, M. M., Zhou, Q. X., Kulis, M. D. *et al.* (2010) Culture techniques and growth characteristics of *Dinophysis acuminata* and its prey. *Chin. J. Oceanol. Limnol.*, **28**, 1230–1239.

Velo Suarez, L., Gonzalez-Gil, S., Pazos, Y. *et al.* (2014) The growth season of *Dinophysis acuminata* in an upwelling system embayment: a conceptual model based on in situ measurements. *Deep-Sea Res. II*, **101**, 141–151.

Yasumoto, T., Murata, M., Oshima, Y. *et al.* (1985) Diarrhetic shellfish toxins. *Tetrahedron*, **41**, 1019–1025.

Yih, W., Kim, H. S., Myung, G. *et al.* (2013) The red-tide ciliate *Mesodinium rubrum* in Korean coastal waters. *Harmful Algae*, **30**, S53–S61.